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Spontaneous Regression Associated With Apoptosis in a Patient With Acute-Type Adult T-Cell Leukemia

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We describe a 76-year-old man with acute-type adult T-cell leukemia, who demonstrated a spontaneous decrease in leukemic cell number, apparently coincident with apoptotic cell death. On admission the patient's white blood cell count was 38.9 × 10^9/l with 77% abnormal lymphocytes. He also had hypoproteinemia (4.3 g/dl) from protein losing enteropathy. After admission the leukemic cell count decreased without chemotherapy, reaching 5.9 × 10^9/l after 2 months. Studies of peripheral lymphocytes demonstrated appearance of the apoptotic cells and DNA ladder formation from the beginning of regression. Same truncated proviral DNA was recognized in primary ATL cells through the whole clinical course. The hypoproteinemia improved with intravenous nutrition, followed by increase of the leukemic cells. This case is the first report that demonstrates tumor-cell apoptosis induced clinical regression in adult T-cell leukemia. Further, we speculate that the hypoproteinemia may have been involved in the leukemic cell apoptosis. Am. J. Hematol. 61:144–148, 1999. © 1999 Wiley-Liss, Inc.

Key words: ATL; apoptosis; spontaneous regression

INTRODUCTION

Adult T-cell leukemia (ATL) is a malignant disease of human T-lymphotropic virus type I (HTLV-I) infected T cells [1]. The clinical picture of ATL is heterogeneous and is usually classified into four categories: acute, chronic, smoldering leukemic-type, and lymphoma-type [2]. Because the mean age of onset is approximately 60 years [2], the process of leukemogenesis must require an extended period after the initial viral infection of the virus. The process of leukemogenesis is thought to involve both clonal expansion and regression. Spontaneous regression has been reported in malignant lymphoid disease. Gattiker et al. have reported 20 cases of spontaneous regression in 209 non-Hodgkin's lymphoma patients [3]. In ATL patients, it has been reported that leukemic cells have the ability to undergo apoptosis, a process thought to play a role in spontaneous regression [4]. However, the relationship between spontaneous regression in ATL patients [5,6] and apoptosis remains obscure. We report a case of acute-type ATL, in which spontaneous regression is apparently coincident with apoptosis.

CASE REPORT

A 76-year-old previously healthy man was admitted to our hospital in January 1994, with 2-months history of leg edema, diarrhea, and lymphadenopathy. On admission, the patient's white blood cell count was 38.9 × 10^9/l; 77% of the cells were abnormal lymphocytes with lobulated nuclei and a CD3, CD4, 25-positive, CD8-negative phenotype. Serum ATLA antibody was positive and monoclonal integration of the human T-lymphotropic virus type I (HTLV-I) genome in leukemic cells was detected by Southern blotting. The serum lactate dehydrogenase (LDH) was 1,766 Wroblewskey units (WU) (normal ≤ 400), and corrected calcium was within normal limits. The patient was diagnosed as acute-type ATL according to the criteria of the Japanese Lymphoma Study Group [2]. The patient was also found to have hy-

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poproteinemia (4.3 g/dl) due to protein losing enteropathy caused by an ulcer of cecum. The etiology of the ulcer was unknown as tissue was not available for pathological studies. After admission the leukemic cell count decreased spontaneously without chemotherapy to a nadir of 5.9×10^9/l and abnormal lymphocytes on peripheral smear were 17% in March 1994. The serum LDH was also reduced to within normal limits and the lymphadenopathy disappeared. The patient's hypoproteinemia accompanied by general edema and ascites progress to 3.2 g/dl, necessitated complete intravenous nutrition, starting in late March. Though the hypoproteinemia and edema improved with 1 month of intravenous nutrition, the abnormal lymphocytes reappeared. The patient received intensive chemotherapy, but succumbed to respiratory failure due to leukemic pulmonary involvement (Fig. 1).

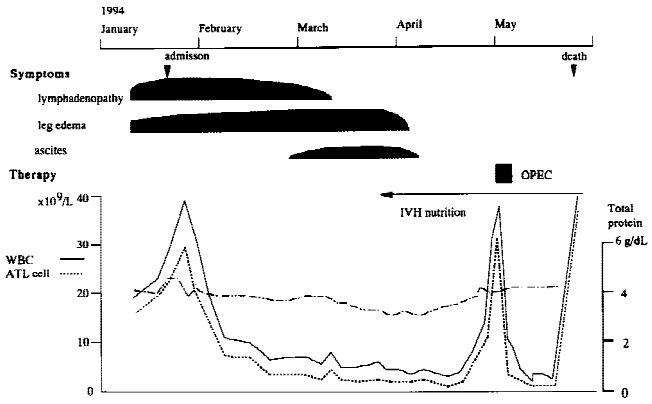

Fig. 1. Clinical course. OPEC, vincristine, etoposide, prednisolone, cyclophosphamide; WBC, white blood cells; ATL, adult T-cell leukemia; IVH, intravenous hydration.

MATERIALS AND METHODS

Cell Separation and Detection of Apoptosis

Peripheral blood mononuclear cells (PBMC) from heparinized blood were separated by density gradient centrifugation. PBMCs were transferred to slides by Cytospin (Shandon Southern Instruments, UK), then underwent May–Giemsa staining to allow for detection of apoptotic cells by light microscopy. Five hundred cells were examined and apoptotic cells (defined by their condensed and fragmented nuclei and decreased cell size) were quantified.

DNA Extraction and Detection of DNA Ladder Formation

DNA was extracted from PBMC by using the phenol/chloroform method. DNA was loaded onto a 1.2% agarose gel containing 0.5% ethidium bromide, electrophoresed at 30 V for 4 hr and visualized by ultraviolet illumination. A sensitive detection assay for ladder formation, 3′-end-label method was performed, according to the manufacturer's instructions (Boehringer, Mannheim, Germany). Briefly, the 1 μg of DNA was 3′-end labeled with digoxigenin by terminal deoxytransferase. DNA was transferred to Nylon membrane (Gene Screen Plus, DuPont/NEN, Boston, MA), after electrophoresis in agarose gel. Then the 3′-end-labeled DNA was visualized by enzyme immunoassay with luminescence method. One μg of DNA extracted from etoposide-treated HL-60, an acute promyelocytic leukemia cell line, was 3′-end labeled for the positive control.

Detection of HTLV-I Proviral DNA

Integrated proviral HTLV-I DNA was detected by Southern blotting analysis, as previously described [7]. Briefly, extracted DNA was digested by restriction enzyme, *Eco*RI or *Pst*I. Then DNA was electrophoresed onto 1% agarose gel, and transferred to Nylon membrane (Gene Screen Plus). DNA dot was hybridized with ^{32}P (111 TBq/mmol; DuPont/NEN) labeled whole HTLV-I proviral DNA.

RESULTS

Typical abnormal lymphocytes with flower-like nuclei were seen, and apoptotic cells detected by using Giemsa staining were not detectable in January. However, they were detectable in March and May 1994, at frequencies of 1.8 and 3.0%, respectively (Fig. 2). Fragmented DNA was observed in March and May 1994, but not detected in January. Faint ladder formation was detected by ethidium bromide staining (Fig. 3A, lanes 4 and 7). Clear ladder formation was demonstrated by digoxigenin 3′-end-label method (Fig. 3B, lanes 3 and 4).

Two bands were detected by Southern blotting in DNA of the ATL cells in January (Fig. 4, lane 3). Intact HTLV-I proviral DNA digested by *Pst*I induces five bands as shown in lane 2. Therefore, the HTLV-I proviral DNA integrated in the ATL cells from this patient is thought to be truncated. The bands of the cells in March were faint as compared with those in January, but, contrarily, the bands in May were intense (Fig. 4, lanes 3, 4, and 6). Because the same-sized two bands were stably detected on *Pst*I-digested DNA, the clonality of leukemic cells was thought to be stable through the whole clinical course (Fig. 4, lanes 3, 4, and 6). The leukemic cells,

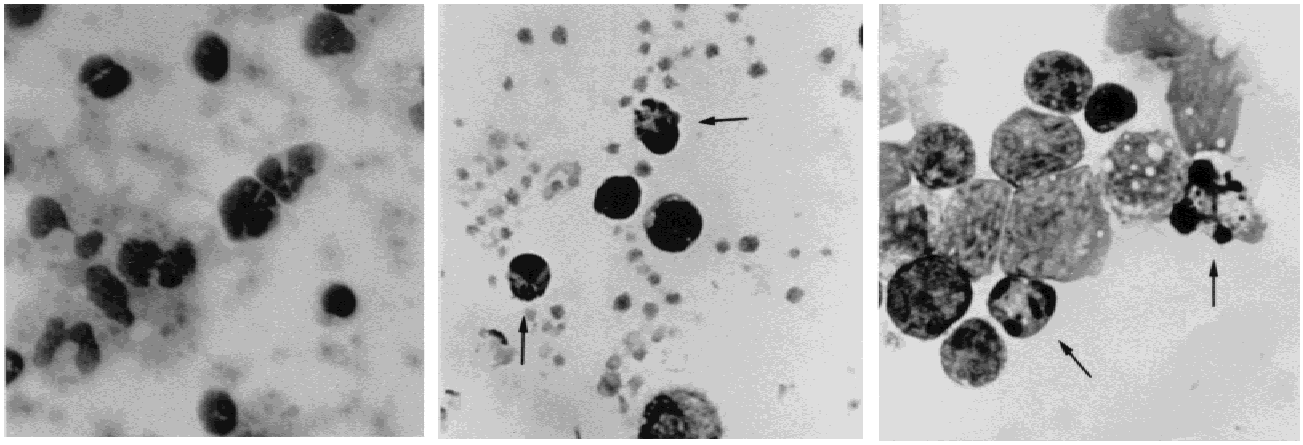

Fig. 2. Morphological demonstration of apoptotic cell death. (A) Primary ATL cell demonstrated by Giemsa staining in January 1994. Apoptotic cells from the patient's peripheral blood cells taken in March 1994 (B) and May 1994 (C), respectively. Arrows indicate apoptotic cells.

which have truncated HTLV-I proviral DNA, was thought to be single clone, as the single band was detected on *Eco*RI-digested DNA (Fig. 4, lane 5).

DISCUSSION

In the present report, we demonstrated that a 2-month period of disease regression was accompanied with apoptosis of ATL cells. Apoptotic cells and DNA-ladder formation were detected after beginning of spontaneous regression but not detected before the regression. Although the DNA-ladder formation was detected clearly, the population of typical apoptotic cells defined morphologically was small in March and May 1994, 1.8% and 3.0%, respectively. The low population of morphological apoptotic cells might be due to morphological characteristics of ATL cell, which have condensed and segmented nuclei. It is likely that we might underestimate the population of morphological apoptotic cells on Giemsa staining. Double staining for CD4 and the Fas antigen showed that CD4 positive cells were also largely positive for Fas antigen on March (data not shown). Therefore, it is likely that a large population of the ATL cells was affected by apoptosis in the regression period. We speculate that the induction of apoptosis may have played a important role in the spontaneous regression by decreasing of primary ATL cell number. The regression was followed by disease recurrence in spite of continuing apoptosis. In May, PBMCs consisted of more than 97% abnormal lymphocytes (data not shown), and apoptotic cells were apparently lymphocytes; therefore, at least a part of primary ATL cells were thought to undergo apoptosis. However, a large population of ATL cells was not undergoing apoptosis or got the ability resisting the signal of apoptosis, and subsequently ATL cells increased in number.

The underlying mechanism of the clinical regression and apoptosis of tumor cells is obscure in the present case. Previous reports suggested that excisional biopsy of lymph node, or infection might trigger spontaneous regression in ATL [6]. However, those are not the case in this patient. Instead, the reduction in ATL cell number was coincident with marked hypoproteinemia. A causal relationship between the hypoproteinemia and apoptosis was unproved. Tsuda et al. reported that interleukin-2 prevents apoptosis of ATL cells induced by serum-free culture *in vitro* [8]. Although the mechanism is not known, marked hypoproteinemia in our case might have induced apoptosis in similar manner to serum free culture induced apoptosis of Tsuda's report. Cytokines, such as TNF-α, have recently been shown to induce apoptosis [9], and interleukin (IL)-1s [10], IL-2 [11], IL-4 [12], and G-CSF [13] may be involved in the growth of ATL cells via inhibition of apoptosis partially. Therefore, it is possible that the marked hypoproteinemia induced apoptosis is mediated by modulating various cytokine levels in the present patient. The regression was followed by recurrence of disease in spite of continuing apoptosis. As previously reported, HTLV-I infected cells revealed expression of Fas, which was followed by apoptosis by anti-Fas antibody stimulating [4]. Although another study of tax transgenic mouse showed that lymphocytes expressing tax protein revealed resistancy to apoptosis [14]. Therefore, the clinical course in the present report imply the possibility that the triggering of apoptosis bring temporally regression but the regression will be followed by recurrence, revealing resistancy to apoptosis.

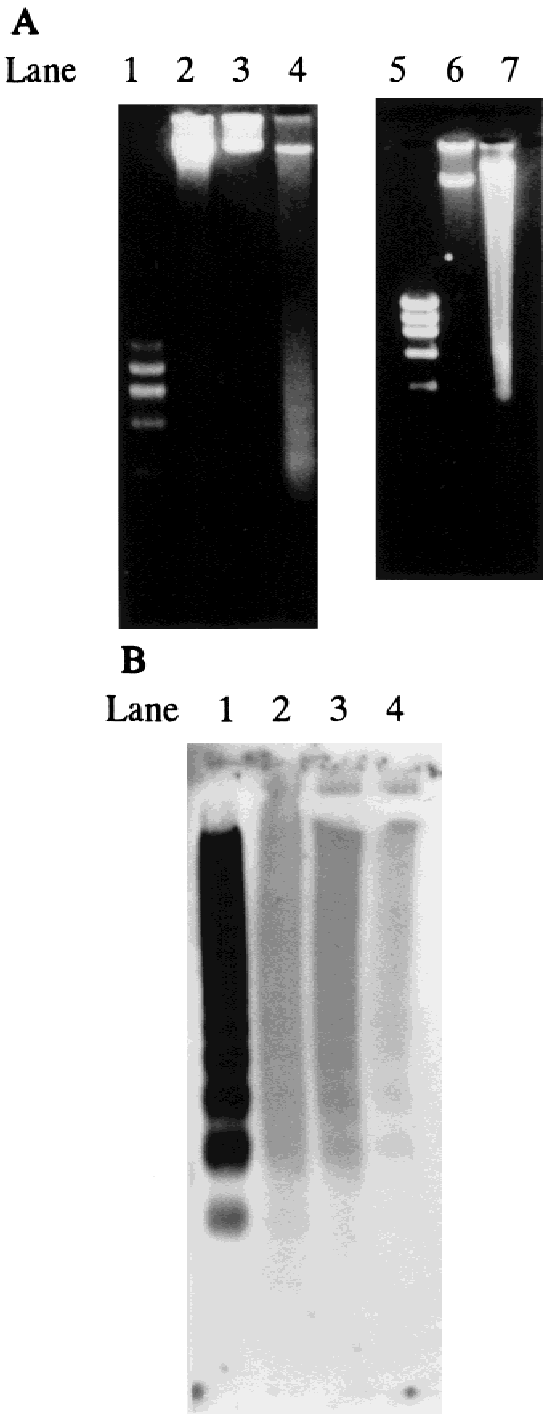

Fig. 3. (A) DNA-ladder formation demonstrated by ethidium bromide staining. Lanes 1 and 5: size-marker DNA, *Hae*III-digested ϕ X 174 DNA, 1357 bp, 1078 bp, 872 bp, 603 bp, 310 bp. Lanes 2 and 6: DNA from healthy donor. Lanes 3, 4, and 7: DNA from the present patients in January, March, and May 1994, respectively. (B) DNA-ladder formation demonstrated by 3′-end-labeling method. Lane 1: DNA from etoposide treated HL-60. Lane 2, 3, and 4: DNA from the present patients in January, March, and May 1994, respectively.

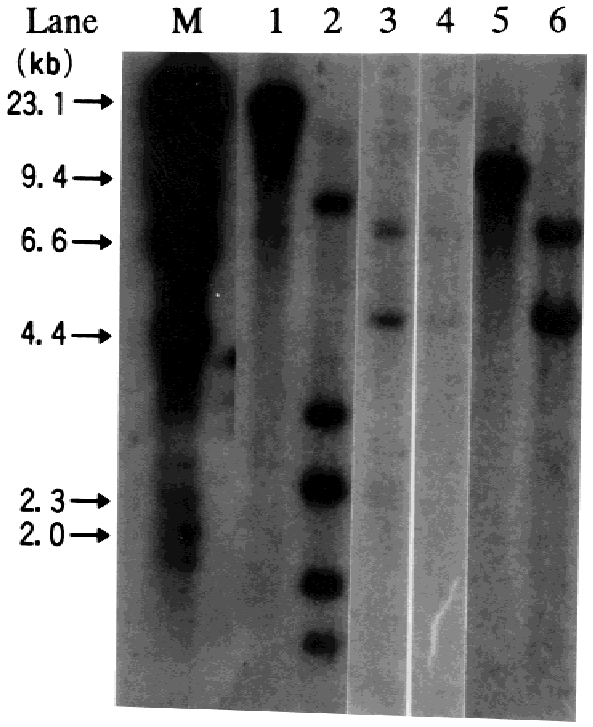

Fig. 4. Southern blotting analysis of HTLV-I in ATL cells, hybridized with ^{32}P-labeled whole HTLV-I proviral DNA. Lane M: size-marker DNA, *Hind*III-digested λ DNA. Lanes 1 and 2: 10 μg of DNA from another ATL patients who has intact HTLV-I; lane 1: digested with *Eco*RI, lane 2: digested with *Pst*I. Lanes 3 and 4: 5 μg of *Pst*I digested DNA from the present patient in January and March 1994. Lanes 5 and 6: 10 mg of DNA from the present patient in May 1994; lane 5: digested with *Eco*RI, lane 5: digested with *Pst*I, lane 6: digested with *Eco*RI, respectively.

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